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Carpels in *Brasenia* (Cabombaceae) are Completely Ascidiate Despite a Long Stigmatic Crest

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• *Background and Aims* The morphological structure of anthetic carpels of *Brasenia* (Cabombaceae), a member of the phylogenetically basal ANITA grade, has not been studied before. The carpel has a long stigmatic crest on the ventral side and could give the impression of a conduplicate structure. This is in contrast to the carpel structure in other genera of the ANITA grade. Therefore, a study of carpel development and carpel structure at anthesis was carried out.
• *Methods* Carpels of *Brasenia schreberi* were studied at different developmental stages up to anthesis by means of microtome section series and SEM to analyse and reconstruct the outer and inner carpel morphology.
• *Key Results* Carpels of *Brasenia* are extremely ascidiate up to anthesis. The elongate stigma originates around the mouth of the young carpel, which is slightly curved toward the centre of the flower. Subsequently, the stigmatic zone below the mouth expands by massive intercalary elongation.
• *Conclusions* In their ascidiate shape, carpels of *Brasenia* are similar to carpels of *Cabomba*, the other genus of Cabombaceae, which, in contrast, has a short stigma restricted to the tip of the carpel. Thus, the morphological structure is independent of the extent (and one-sidedness) of the stigma. The outer shape of carpels at anthesis does not allow the inference of the inner morphological surface. If an angiosperm carpel has a one-sided stigma it can be extremely conduplicate or extremely ascidiate. Therefore, caution has to be used in the interpretion of the structure of fossil carpels.

Key words: *Brasenia*, *Cabomba*, Cabombaceae, Nymphaeaceae, basal angiosperms, carpel structure.

INTRODUCTION

The carpels of *Brasenia*, a monotypic genus in Cabombaceae (Nymphaeales) have a puzzling combination of features. They are known to be ascidiate at least in early development (Schneider *et al*., 2003). In contrast, at anthesis they are conspicuously one-sided, having a long stigma on the ventral side, which has been described as a crest.

Although young carpels of *Brasenia* have been illustrated with their complete outer and inner morphological surfaces (Troll, 1933), anthetic carpels have never been analysed and discussed as to their external and internal morphology. Only surface views of anthetic carpels were illustrated (Osborn and Schneider, 1988), and a longitudinal section of an anthetic carpel but without showing the complete morphological surface in the critical upper zone (Ito, 1986).

From the earlier accounts, the morphological structure of carpels at anthesis is not clear: ‘the dorsal side is slightly longer than the ventral so that the mouth of the carpel is displaced to the ventral side, also the elongate stigma is on the side’ (Troll, 1933, in translation); ‘the carpel is horseshoe shaped, unites with its margins’ (Khanna, 1965); the carpel ‘is ascidial in shape throughout most of its development’ (Richardson, 1969); the carpel has a ‘long stigma’ (Ito, 1986); carpels have ‘linear stigmas with abaxial stigmatic crests’ (Osborn and Schneider, 1988); carpels have a ‘linear, extremely papillate, stigma’ (Williamson and Schneider, 1993); ‘young carpel primordia are ascidiate’ (Schneider *et al*., 2003).

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Thus, the combination of a carpel that is completely ascidiate at least in early development and a long stigmatic crest at anthesis has not been discussed in detail with respect to its structural consequences. It seemed important to carry out this study because, in the literature, carpels with one-sided stigmas have sometimes been described uncritically as conduplicate. Caution is especially necessary in the interpretation of carpels in fossil flowers, in which the stigma is not even always distinctive (e.g. *Archaefructus*, Sun *et al*., 2002; discussion in Friis *et al*., 2003). The present study aims to elucidate the morphological structure of the carpels of *Brasenia*.

MATERIALS AND METHODS

Flowers and flower buds at various developmental stages fixed in 70 % ethanol of *Brasenia schreberi* J.F. Gmel. [Rolf Rutishauser, *s.n.*, coll. July 2001, Elliott Lake, Ontario, Canada (flower buds); Ed Schneider, *s.n.*, coll. 27 June 1997, Texas, USA (open flowers)] were studied with microtome section series and with scanning electron microscopy (SEM). For microtome sectioning, specimens were embedded in Kulzer’s Technovit (2-hydroethyl methacrylate) and sectioned with a Microm HM 355 rotary microtome and a conventional microtome knife D. The 10-μm-thick sections were stained with ruthenium red and toluidine blue. For SEM studies, specimens were post-fixed in 2 % osmium tetroxide, dehydrated in ethanol and acetone, critical-point dried and sputter-coated with gold, and observed in a Hitachi S-4100 at 20 kV. The SEM micrographs were electronically recorded.

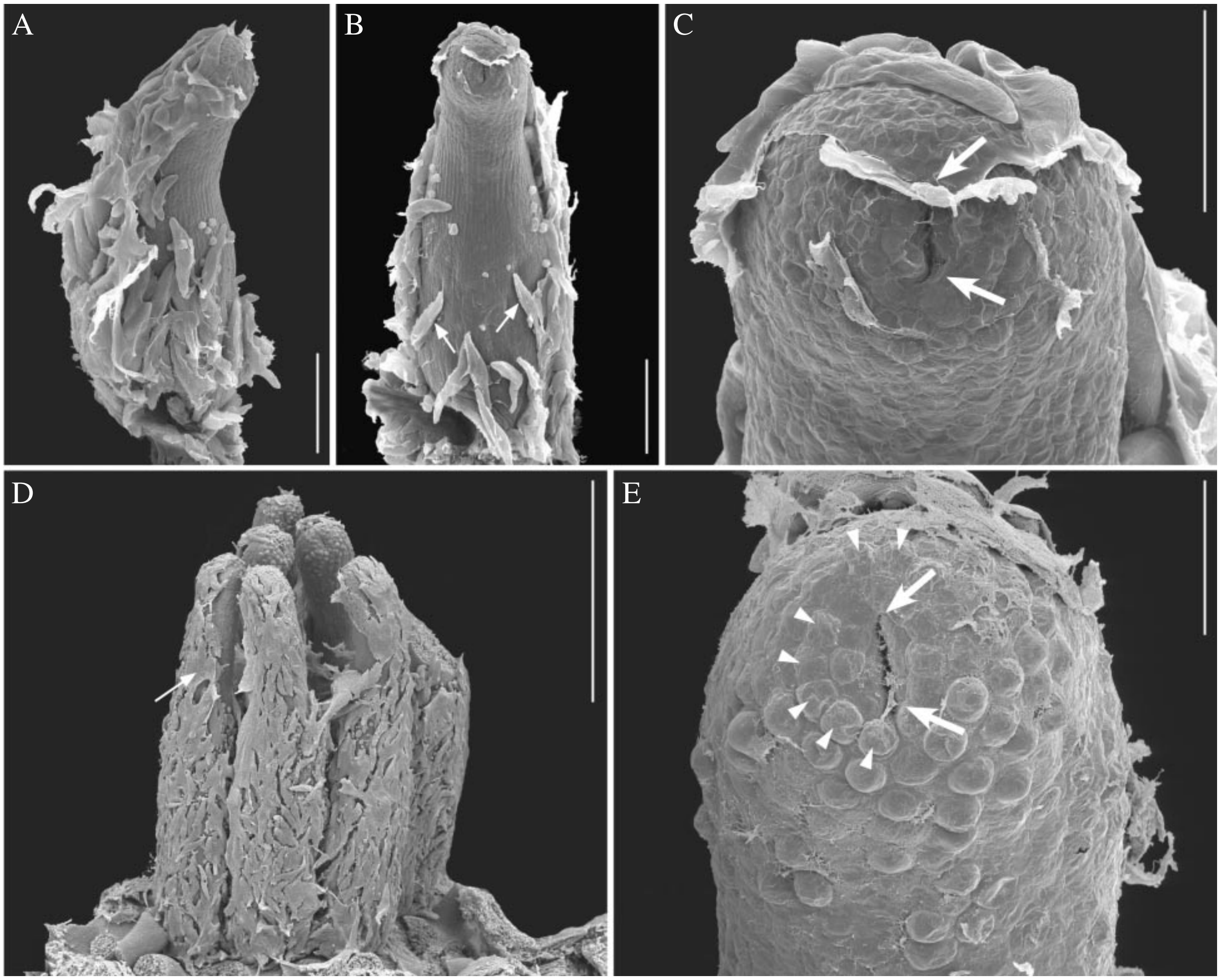

FIG. 1. *Brasenia schreberi*. (A–C) Carpel from young floral bud. Stage before beginning of stigma differentiation. (A) From the side, ventral on the right. (B) From ventral, two hairs marked with arrows; only large apical cell of each hair visible, the two basal cells are much smaller and hidden. (C) Close-up of tip of B, extent of mouth of carpel indicated by arrows. (D and E) Carpels from slightly older bud, stigma differentiation beginning. (D) Group of carpels of a bud, dorsal parts covered by secretory hairs and secretion (arrow points to secretion). (E) Close-up of tip of one of the carpels of (D), incipient stigmatic papillae visible (arrowheads), extent of mouth of carpel indicated by arrows. Scale bars: A and B = 200 µm; C and E = 100 µm; D = 1 mm.

RESULTS

Carpel development

The carpels develop as straight tubes at the beginning with the mouth at the tip and directed upward. Carpels in medium-sized floral buds have the tip slighly curved toward the ventral side. In young stages the carpels are covered by mucilage-secreting hairs, especially on the dorsal side. The mucilage more or less covers the carpels on the dorsal side (Fig. 1). The hairs are three-cellular with two short basal cells and a large apical cell, which is elongate in the direction parallel to the carpel. The mucilage is probably secreted by this apical cell. Such three-cellular hairs are continuously produced during carpel growth in bud but toward anthesis less mucilage appears to be present (Fig. 2). The unicellular stigmatic papillae appear much later than the three-cellular hairs, at first around the mouth of the carpel (Fig. 1E), and then in a band that elongates by intercalary growth of the upper part of the carpel (Fig. 2). Carpel development in the later bud is characterized by an excessive elongation of the stigmatic zone below the mouth, whereas the ovary grows much less (compare Fig. 2A and B with 2D and E). During this developmental phase the dense cover of secretory hairs on the dorsal side of the carpel thins out.

Carpels at anthesis

Anthetic carpels of *Brasenia schreberi* are approx. 8–9 mm long and slender. The stigma is 5 mm long, the ovary 3 mm, and there is a short stalk (Figs 2D and E and 3A–V). The stigma consists of a long band of receptive tissue along the ventral side of the carpel, covered by long unicellular papillae. The stigma extends from the tip downwards for more than half the length of the carpel. In fact, the stigma is present not only on the ventral half of the circumference of the carpel but extends over the flanks and occupies over 300° of the carpel circumference

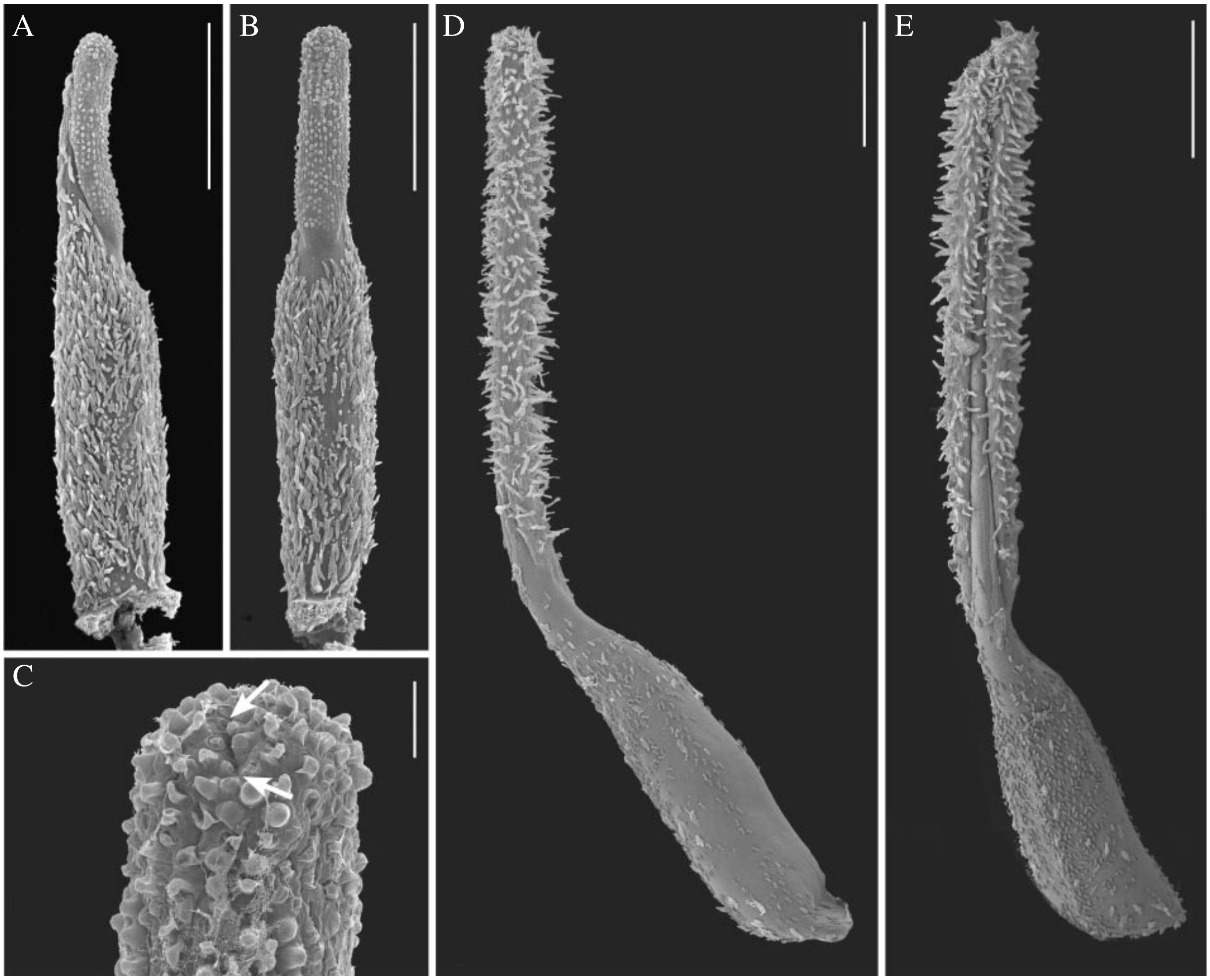

FIG. 2. *Brasenia schreberi*. (A–C) Carpel from advanced floral bud. (A) From the side, ventral on the right. (B) From ventral. (C) Close-up of tip of (B), extent of mouth of carpel indicated by arrows. (D and E) Carpel in early female phase of anthesis. (D) From the side, ventral on the right. (E) From dorsal. Scale bars: A, B, D and E = 1 mm; C = 100 µm.

but is lacking in the dorsal median area of the carpel (Figs 2E, 3A, D–K and W and 4A–C). The stigma is especially expanded in its upper part (Figs 3D–H and 4A) and narrows toward the base, where it occupies only about half the circumference of the carpel (180°) or less (Figs 3I–K and 4B). The stigma appears inflated because its tissue is much larger-celled than the non-stigmatic area on the dorsal side and in the centre of the carpel as seen in transverse section (Fig. 4A). The mouth of the carpel is not easily visible when viewed from the surface at anthesis, because it is hidden by the stigmatic papillae. It is best seen in a transverse microtome section series. The mouth is on the ventral side almost at the tip of the carpel; it is only a very short (approx. 0·1 mm long) longitudinal slit (Fig. 3A and C–E). The entire inside of the carpel is filled with a secretion. There is no post-genital fusion of the inner surface, not even at the mouth. Between the stigma and ovary there is a short transitional zone, a short style, in which the internal space becomes wider toward the ovary (Fig. 3L and M). The ovary commonly contains two anatropous, syntropous (i.e. curved away from the rim of the carpel; Endress, 1994), pendant ovules, one above the other, on a dorsal–median placenta (Fig. 3A and N–T). If a third ovule is present, it is obliquely directed and its placenta is lateral.

The main carpel vascular bundle is a median dorsal bundle, which extends up to the level of the mouth (Figs 3D–V and 4A and B). The vascular bundle of each of the two ovules connects with this dorsal bundle (Fig. 3N and R). At the level at which the bundle of the upper ovule connects with the carpel dorsal bundle, two lateral carpel bundles also depart from the dorsal bundle and extend along the ovary downwards to the carpel base (Fig. 3N–V). This is repeated at the connection level of the vascular bundle of the lower ovule. These two lower lateral bundles are somewhat weaker; they connect lower down with the other lateral bundles (Fig. 3R and S). In the carpel stalk the two lateral bundles join each other, forming a ventral bundle, which then joins the dorsal bundle (Fig. 3V). All vascular bundles are collateral.

The stigmatic tissue is large-celled (Fig. 4A). Cell files of obliquely elongate cells extend from the stigmatic surface inwards and downwards (first illustrated by Chifflot, 1902), as is common in fountain-shaped pollen tube transmitting tissues (Figs 3W and 4C). The inner morphological surface,

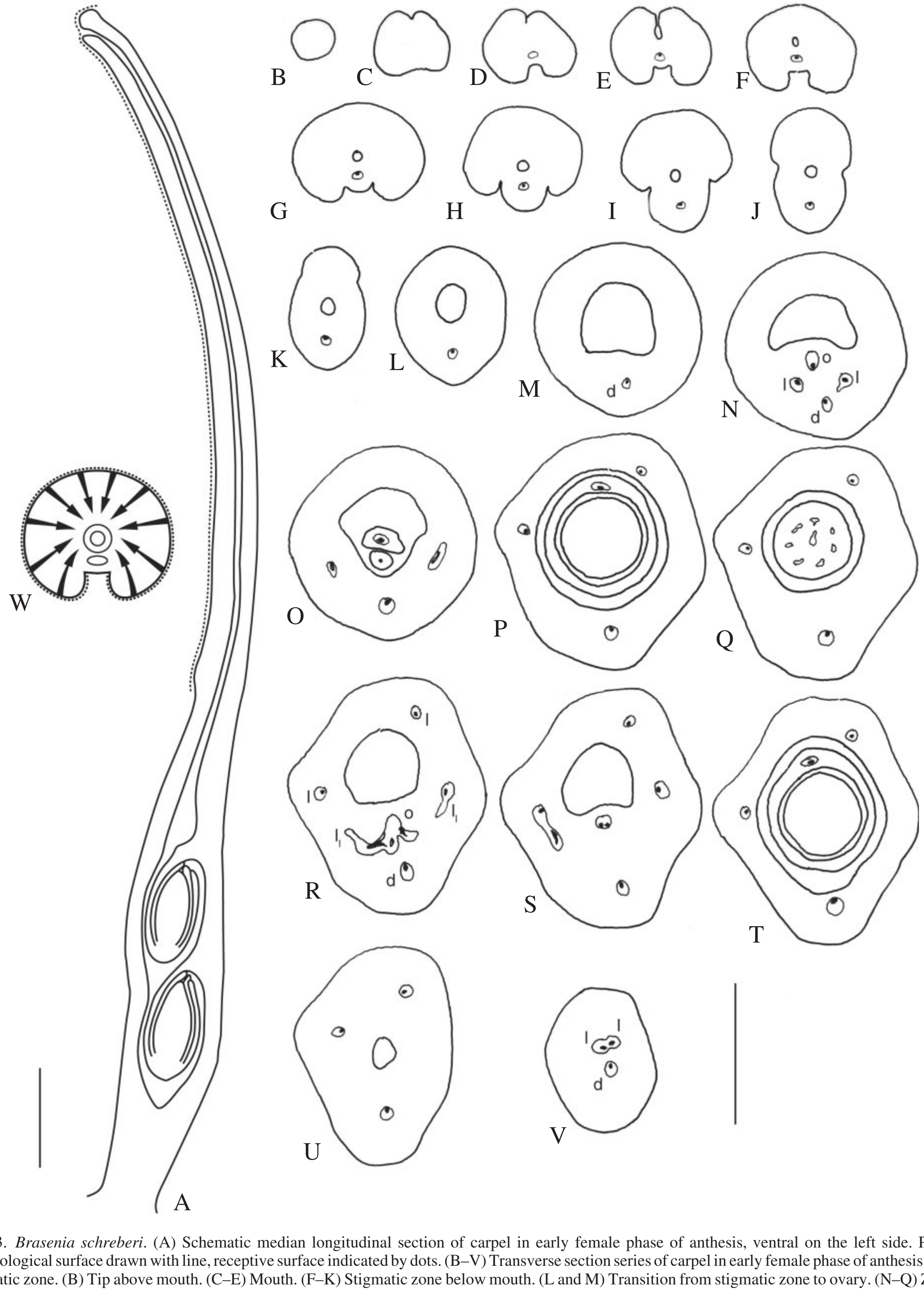

FIG. 3. *Brasenia schreberi*. (A) Schematic median longitudinal section of carpel in early female phase of anthesis, ventral on the left side. Primary morphological surface drawn with line, receptive surface indicated by dots. (B–V) Transverse section series of carpel in early female phase of anthesis. (B–K) Stigmatic zone. (B) Tip above mouth. (C–E) Mouth. (F–K) Stigmatic zone below mouth. (L and M) Transition from stigmatic zone to ovary. (N–Q) Zone of upper ovule. (R–T) Zone of lower ovule. (U) Ovary base. (V) Stalk. (W) Schematic transverse section of anthetic carpel in stigmatic region. Arrows indicate the direction of pollen tube growth inward and downward, receptive surface indicated by dots. d, Dorsal vascular bundle; l, lateral bundle; l_l, lower lateral bundle; o, ovule bundle. Scale bars: A = 1 mm, B–V = 500 µm.

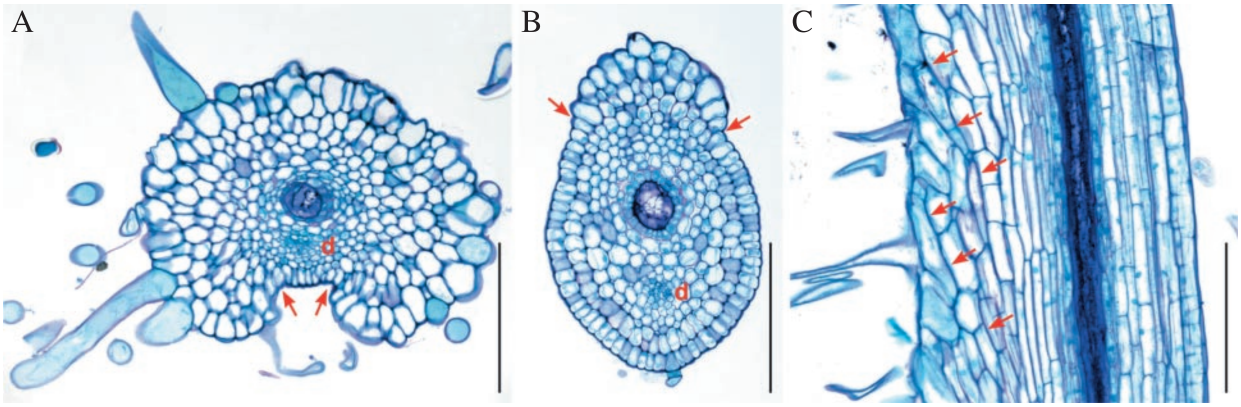

FIG. 4. *Brasenia schreberi*. Transverse sections of a carpel at anthesis. (A) Upper stigmatic region. (B) Base of the stigmatic region. Red arrows in A and B indicate the border of the receptive surface. (C) More or less median longitudinal section of a carpel at anthesis in stigmatic zone (vascular bundle not in the section). The oblique cell files that are directed inward and downward and determine the direction of pollen tube growth are indicated with red arrows. The inner space of the carpel, delimited by the inner part of the primary morphological surface, is filled with mucilaginous secretion (dark). The mucilage is secreted by the inner epidermis, which consists of cytoplasm-rich cells. The cell layers between the oblique cell files and the inner epidermis consist of much elongated cells. d, Dorsal vascular bundle. Scale bars = 200 µm.

which surrounds the central canal is formed by an epidermis layer of small, cytoplasm-dense and relatively large-nucleate cells, which produce the mucilaginous secretion in the canal (Fig. 4). This innermost cell layer is surrounded by about two layers of narrow cells, which are elongate in longitudinal direction. The oblique cell files converge toward these longitudinally directed cell files (Fig. 4C). This system of obliquely and longitudinally directed cell files constitutes the pollen tube transmitting tract. It remains to be studied exactly how the pollen tubes reach the micropyle of the ovules. The tissue of the ovary is excessively rich in intercellular spaces. Only the outermost two or three cell layers and the inner epidermis remain compact at anthesis.

DISCUSSION

Young carpels of *Brasenia* and *Cabomba* are both completely ascidiate, forming a narrow tube, with the small mouth of the tube at the tip. Their similarity is obvious from SEM illustrations for *Brasenia* (Schneider *et al.*, 2003; this study) and *Cabomba* (Tucker and Douglas, 1993; Endress, 2001). At anthesis the overall shape is still the same, consisting of a long tube with the inner space ending exactly (*Cabomba*) or almost (*Brasenia*) at the tip. The obvious difference between *Brasenia* and *Cabomba* is not in morphology but in histology. In *Cabomba* the stigma is small and restricted to the carpel tip, embracing the mouth. In *Brasenia* the stigma also embraces the mouth but extends from the tip downward for more than half the length of the carpel on the ventral side. The long stigma of *Brasenia* is correlated with wind pollination (Osborn and Schneider, 1988), whereas *Cabomba* with the small stigma is insect-pollinated (Schneider and Jeter, 1982). Another difference is that in *Brasenia* the two ovules (if two are present, which is the normal case) are always in a line one upon the other exactly on the dorsal side, whereas in *Cabomba* the position of the ovules is more variable; however, dorsal placenta may also occur (e.g. Strasburger, 1879; Troll, 1933; Moseley *et al.*, 1984; Ito, 1986). Variability in ovule position and carpel vascularization of *Brasenia* was discussed by Saunders (1936) and Richardson (1969).

Carpels of plants of the ANITA grade (Qiu *et al.*, 1999), Amborellaceae, Nymphaeales and Austrobaileyales, the basal-most angiosperms, predominantly have extremely ascidiate carpels (Amborellaceae: Endress and Igersheim, 1997, 2000*a*, *b*; Endress, 2001; Posluszny and Tomlinson, 2003; Buzgo *et al.*, 2004; Nymphaeales: e.g. Tucker and Douglas, 1993; Igersheim and Endress, 1998; Endress, 2001; Schneider *et al.*, 2003; Austrobaileyales: Leinfellner, 1966; Endress, 1980, 1983; Endress and Sampson, 1983; van Heel, 1983; Tucker and Bourland, 1994; Endress, 2001). Such ascidiate carpels may be ancestral for extant angiosperms (Doyle and Endress, 2000; Endress and Igersheim, 2000*a*). Ascidiate form results in an only relatively short mouth of the carpel, that is the region of transition of the primary morphological surface from the outside to the inside of the organ.

Strongly ascidiate carpels tend to be correlated with sealing by secretion (and not post-genital fusion) of the inner morphological surfaces. This is the case in the majority of extant basal-most angiosperms (ANITA grade). There is also a tendency for heavy secretion within carpels or syncarpous gynoecia in water plants or plants of otherwise moist habitats. The majority of extant basal-most angiosperms live in such habitats (Feild *et al.*, 2004). But heavy secretion is also present in a number of other plant groups of such habitats (Endress, 1990, 2004; Rudall *et al.*, 1998; Kocyan and Endress, 2001).

Extremely ascidiate carpels form a contrast to extremely conduplicate carpels, in which the mouth forms a long ventral slit, and the stigma may extend as two crests along the ventral slit, or it may be restricted to the tip of the carpel. Such extremely conduplicate carpels are present in some other basal angiosperms, e.g. Magnoliales, such as Annonaceae and Myristicaceae. If the two crests in conduplicate carpels are stigmatic all along, they are especially prominent (e.g. *Tasmannia*, Winteraceae, Canellales). If the stigma is restricted to the carpel tip, the crests are less conspicuous (e.g. Myristicaceae, Magnoliales). Thus, the extension of the stigma and the morphological structure of the carpel are more or less independent from each other. Also the shape of the placenta is more or less independent of the morphological structure of the carpel. In an extremely ascidiate carpel the placenta may be in two lines at the ventral side, exactly as in a conduplicate carpel (e.g. Austrobaileyaceae; Endress, 1980), or it may be single and median at the ventral base of the carpel (e.g. Potamogetonaceae; Eber, 1934; Igersheim *et al*., 2001), or in a median line on the dorsal side, as in *Brasenia*.

In extremely ascidiate carpels, commonly, the stigma is developed around the short mouth of the carpel. The combination of extremely ascidiate carpels with a long, ventral stigma, as in *Brasenia*, is unusual, but it is a form that has to be taken into consideration as potentially present in other, extinct, basal angiosperms. As discussed earlier, *Brasenia* is wind-pollinated, and the long stigma, which is covered by long papillae, is likely an adaptation to wind-pollination (Osborn and Schneider, 1988).

Thus, a long, slender carpel with a long stigma on its ventral side can be extremely conduplicate (as, e.g., in *Cercidiphyllum*; Endress, 1986) or extremely ascidiate (as in *Brasenia*; this study). The morphology of such a carpel can only be reconstructed by an analysis with microtome section series, as was carried out in this study.

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